

Journal of General Virology, Vol 76, 1729-1736, Copyright © 1995 by
Society for General Microbiology

ARTICLES

In vitro cleavage of hepatitis C virus polyprotein substrates by purified recombinant NS3 protease

ED D'Souza, K Grace, DV Sangar, DJ Rowlands and BE Clarke

Biology Division, Wellcome Research Laboratories, Beckenham, Kent, UK.

The non-structural protein NS3 of hepatitis C virus has been expressed in bacteria as a polyhistidine fusion protein which can be produced in a soluble form and easily purified by affinity chromatography. Using an in vitro transcription and translation system we have been able to demonstrate that this protein can proteolytically process substrate molecules derived from the non-structural region of the polyprotein. Using this assay system we have been able to optimize basic biochemical characteristics of the purified enzyme. Parallel experiments show that the full-length NS3 protein also possesses ATPase activity, indicating the bifunctional nature of the protein. In contrast, purified NS3 in which the predicted catalytic serine has been mutated loses protease but retains ATPase activity.

This article has been cited by other articles:

- Steinkühler, C., Tomei, L., De Francesco, R. (1996). In Vitro Activity of Hepatitis C Virus Protease NS3 Purified from Recombinant Baculovirus-infected Sf9 Cells. *J. Biol. Chem.* 271: 6367-6373 [Abstract] [Full Text]
- Urbani, A., Bianchi, E., Narjes, F., Tramontano, A., De Francesco, R., Steinkühler, C., Pessi, A. (1997). Substrate Specificity of the Hepatitis C Virus Serine Protease NS3. *J. Biol. Chem.* 272: 9204-9209 [Abstract] [Full Text]

RECOMBINANT
NS3 FUSION
2 Refs → claim 1, 2, 3

(1)

RECOMBINANT
FUSION

(2)

Journal of General Virology

[HOME](#) [HELP](#) [FEEDBACK](#) [SUBSCRIPTIONS](#) [ARCHIVE](#) [SEARCH](#) [TABLE OF CONTENTS](#)

Journal of General Virology, Vol 76, 3021-3029, Copyright © 1995 by
Society for General Microbiology

ARTICLES

In vivo and in vitro trans-cleavage activity of hepatitis C virus serine proteinase expressed by recombinant baculoviruses

T Suzuki, M Sato, S Chieda, I Shoji, T Harada, Y Yamakawa, S Watabe, Y Matsuura and T Miyamura

Department of Virology II, National Institute of Health, Tokyo, Japan.

By the use of recombinant baculoviruses, the trans-cleavage of hepatitis C virus (HCV) non-structural polyprotein was studied. The viral serine proteinase encoded by the NS3 gene was expressed efficiently in insect cells infected with a baculovirus recombined with HCV cDNA corresponding to amino acids 1046-1243 and the signal sequence of the rabies virus G protein. Coinfection studies showed the in vivo trans-cleavage activity of the expressed protein by the use of a recombinant producing NS5 as a substrate. We also found that the partially purified NS3 serine proteinase prepared from the recombinant- infected cells could cleave NS5A/5B substrate. Characterization of the proteinase obtained wil provide basic knowledge on processing of the HCV polyprotein.

recombinant
NS3
fusion

This article has been cited by other articles:

- Aizaki, H., Aoki, Y., Harada, T., Ishii, K., Suzuki, T., Nagamori, S., Toda, G., Matsuura, Y., Miyamura, T. (1998). Full-Length Complementary DNA of Hepatitis C Virus Genome From an Infectious Blood Sample. *Hepatology* 27: 621-627 [\[Abstract\]](#) [\[Full Text\]](#)
- Ishii, K., Tanaka, Y., Yap, C.-C., Aizaki, H., Matsuura, Y., Miyamura, T. (1999). Expression of Hepatitis C Virus NS5B Protein: Characterization of Its RNA Polymerase Activity and RNA Binding. *Hepatology* 29: 1227-1235 [\[Abstract\]](#) [\[Full Text\]](#)
- Urbani, A., Bianchi, E., Narjes, F., Tramontano, A., De Francesco, R., Steinkühler, C., Pessi, A. (1997). Substrate Specificity of the Hepatitis C Virus Serine Protease NS3. *J. Biol. Chem.* 272: 9204-9209 [\[Abstract\]](#) [\[Full Text\]](#)

[HOME](#) [HELP](#) [FEEDBACK](#) [SUBSCRIPTIONS](#) [ARCHIVE](#) [SEARCH](#) [TABLE OF CONTENTS](#)

National Library
of Medicine09/600 493
PubMed

PubMed Nucleotide Protein Genome Structure PopSet Taxonomy OMIM

Search PubMed ☒ for DNA Vaccine AND HCV Go Clear

Limits Preview/Index History Clipboard

Entrez PubMed

Display Summary ☒ Save Text Order Details Add to Clipboard

Show: 20 ☒ Items 1-20 of 34 Page 1 of 2 Select page:

PubMed Services

- ☐ 1: Forns X, Payette PJ, Ma X, Satterfield W, Eder G, Mushahwar IK, Govindarajan S, Davis HL, Emerson SU, Purcell RH, Bukh J. Related Ai

Vaccination of chimpanzees with plasmid DNA encoding the hepatitis virus (HCV) envelope E2 protein modified the infection after challenge with homologous monoclonal HCV.

Hepatology. 2000 Sep;32(3):618-25.

PMID: 10960458; UI: 20417952

NEW

- ☐ 2: Girard M. Related Ai

[Antiviral vaccines].

Med Trop (Mars). 1999;59(4 Pt 2):522-6. French.

PMID: 10901858; UI: 20360315

Related Resources

- ☐ 3: Kamei A, Tamaki S, Taniyama H, Takamura S, Nishimura Y, Kagawa Y, Uno-Furuta S, Kaito M, Kim G, Toda M, Matsuura Y, Miyamura T, Adachi Y, Yasutomi Y. Related Ai

Induction of hepatitis C virus-specific cytotoxic T lymphocytes in mice an intrahepatic inoculation with an expression plasmid.

Virology. 2000 Jul 20;273(1):120-6.

PMID: 10891414; UI: 20351730

- ☐ 4: Heile JM, Fong YL, Rosa D, Berger K, Saletti G, Campagnoli S, Bensi G, Capo S, Coates S, Crawford K, Dong C, Wininger M, Baker G, Cousens L, Chien D, Ng P, Archangel P, Grandi G, Houghton M, Abrignani S. Related Ai

Evaluation of hepatitis C virus glycoprotein E2 for vaccine design: an endoplasmic reticulum-retained recombinant protein is superior to secreted recombinant protein and DNA-based vaccine candidates.

J Virol. 2000 Aug;74(15):6885-92.

PMID: 10888628; UI: 20347351

- ☐ 5: Pancholi P, Liu Q, Tricoche N, Zhang P, Perkus ME, Prince AM. Related Ai

DNA prime-canarypox boost with polycistronic hepatitis C virus (HCV) genes generates potent immune responses to HCV structural and nonstructural proteins.

J Infect Dis. 2000 Jul;182(1):18-27.

PMID: 10882577; UI: 20341727

- ☐ 6: Shan M, Liu K, Fang H. Related Ai

[DNA vaccination of the induction of immune responses by codelivery IL-12 expression vector with hepatitis C structural antigens].

②

Chung Hua Kan Tsang Ping Tsa Chih. 1999 Dec;7(4):236-9. Chinese.
PMID: 10715797; UI: 20180687

- ☐ **7:** [Lee AY, Polakos NK, Otten GR, Ulmer JB, Houghton M, Paliard X.](#) Related Ar
Quantification of the number of cytotoxic T cells specific for an immunodominant HCV-specific CTL epitope primed by DNA immunization.
Vaccine. 2000 Mar 17;18(18):1962-8.
PMID: 10699347; UI: 20165121
- ☐ **8:** [Song MK, Lee SW, Suh YS, Lee KJ, Sung YC.](#) Related Ar
Enhancement of immunoglobulin G2a and cytotoxic T-lymphocyte responses by a booster immunization with recombinant hepatitis C virus E2 protein in E2 DNA-primed mice.
J Virol. 2000 Mar;74(6):2920-5.
PMID: 10684312; UI: 20148989
- ☐ **9:** [Arichi T, Saito T, Major ME, Belyakov IM, Shirai M, Engelhard VH, Feinstone SM, Berzofsky JA.](#) **Free in PMC** Related Ar
Prophylactic DNA vaccine for hepatitis C virus (HCV) infection: HCV-specific cytotoxic T lymphocyte induction and protection from HCV-recombinant vaccinia infection in an HLA-A2.1 transgenic mouse model.
Proc Natl Acad Sci U S A. 2000 Jan 4;97(1):297-302.
PMID: 10618412; UI: 20087243
- ☐ **10:** [Gordon EJ, Bhat R, Liu Q, Wang YF, Tackney C, Prince AM.](#) Related Ar
Immune responses to hepatitis C virus structural and nonstructural proteins induced by plasmid DNA immunizations.
J Infect Dis. 2000 Jan;181(1):42-50.
PMID: 10608749; UI: 20086996
- ☐ **11:** [Houghton M.](#) Related Ar
Strategies and prospects for vaccination against the hepatitis C virus.
Curr Top Microbiol Immunol. 2000;242:327-39. Review. No abstract available.
PMID: 10592667; UI: 20060252
- ☐ **12:** [Nishimura Y, Kamei A, Uno-Furuta S, Tamaki S, Kim G, Adachi Y, Kuribayashi K, Matsuura Y, Miyamura T, Yasutomi Y.](#) Related Ar
A single immunization with a plasmid encoding hepatitis C virus (HCV) structural proteins under the elongation factor 1-alpha promoter elicits HCV-specific cytotoxic T-lymphocytes (CTL).
Vaccine. 1999 Nov 12;18(7-8):675-80.
PMID: 10547427; UI: 20016519
- ☐ **13:** [Encke J, zu Putlitz J, Wands JR.](#) Related Ar
DNA vaccines.
Intervirology. 1999 Sep;42(2-3):117-24. Review.
PMID: 10516466; UI: 99447701
- ☐ **14:** [Irshad M.](#) Related Ar
DNA-vaccines against hepatitis B and C viral infections: a brief overview.

③

Trop Gastroenterol. 1999 Apr-Jun;20(2):64-7. Review. No abstract available.
PMID: 10484889; UI: 99414530

- ☐ **15:** Vidalin O, Tanaka E, Spengler U, Trepo C, Inchauspe G. Related A
Targeting of hepatitis C virus core protein for MHC I or MHC II presentation does not enhance induction of immune responses to DN vaccination.
DNA Cell Biol. 1999 Aug;18(8):611-21.
PMID: 10463057; UI: 99392353
- ☐ **16:** Hu GJ, Wang RY, Han DS, Alter HJ, Shih JW. Related A
Characterization of the humoral and cellular immune responses again hepatitis C virus core induced by DNA-based immunization.
Vaccine. 1999 Aug 6;17(23-24):3160-70.
PMID: 10462252; UI: 99389348
- ☐ **17:** Fournillier A, Depla E, Karayiannis P, Vidalin O, Maertens G, Trepo C, Inchauspe G. Related A
Expression of noncovalent hepatitis C virus envelope E1-E2 complex not required for the induction of antibodies with neutralizing properties following DNA immunization.
J Virol. 1999 Sep;73(9):7497-504.
PMID: 10438839; UI: 99370193
- ☐ **18:** Forns X, Emerson SU, Tobin GJ, Mushahwar IK, Purcell RH, Bukh J. Related A
DNA immunization of mice and macaques with plasmids encoding hepatitis C virus envelope E2 protein expressed intracellularly and on cell surface.
Vaccine. 1999 Apr 9;17(15-16):1992-2002.
PMID: 10217599; UI: 99231951
- ☐ **19:** Cho JH, Lee SW, Sung YC. Related A
Enhanced cellular immunity to hepatitis C virus nonstructural proteins codelivery of granulocyte macrophage-colony stimulating factor gene intramuscular DNA immunization.
Vaccine. 1999 Mar 5;17(9-10):1136-44.
PMID: 10195625; UI: 99210000
- ☐ **20:** Inchauspe G. Related A
DNA vaccine strategies for hepatitis C.
J Hepatol. 1999 Feb;30(2):339-46. Review. No abstract available.
PMID: 10068118; UI: 99165487

Display	Summary	<input type="checkbox"/>	Save	Text	Order	Details	Add to Clipboard
Show:	20	<input type="checkbox"/>	Items 1-20 of 34		Page 1 of 2		Select page:

Write to the Help Desk
NCBI | [NLM](#) | [NIH](#)
[Department of Health & Human Services](#)
[Freedom of Information Act](#) | [Disclaimer](#)

National Library
of Medicine

PubMed

4

PubMed	Nucleotide	Protein	Genome	Structure	PopSet	Taxonomy	OMIM
Search	PubMed	<input checked="" type="checkbox"/> for	DNA Vaccine AND HCV			Go	Clear
		Limits	Preview/Index	History	Clipboard		

Entrez PubMed

Display	Summary	<input checked="" type="checkbox"/>	Save	Text	Order	Details	Add to Clipboard
Show:	20	<input checked="" type="checkbox"/>	Items 21-34 of 34		Page 2 of 2		Select page:

PubMed Services

- ☐ **21:** Papa S, Rinaldi M, Mangia A, Parrella P, Signori E, Lombardi L, Fazio Related Ar
VM.

Development of a multigenic plasmid vector for HCV DNA immunizati
Res Virol. 1998 Sep-Oct;149(5):315-9.
PMID: 9879611; UI: 99095684

PATENT
Authors
NEW

Related Resources

- ☐ **22:** Encke J, zu Putlitz J, Geissler M, Wands JR. Related Ar

Genetic immunization generates cellular and humoral immune respor
against the nonstructural proteins of the hepatitis C virus in a murine
model.

J Immunol. 1998 Nov 1;161(9):4917-23.
PMID: 9794426; UI: 99008557

- ☐ **23:** Qu D, Yuan ZH, He LF, Yang L, Li GD, Wen YM. Related Ar

Effect of plasmid DNA on immunogenicity of HBsAg-anti-HBs comple
Viral Immunol. 1998;11(2):65-72.
PMID: 9765028; UI: 98436057

- ☐ **24:** Lee SW, Cho JH, Sung YC. Related Ar

Optimal induction of hepatitis C virus envelope-specific immunity by
bicistronic plasmid DNA inoculation with the granulocyte-macrophage
colony-stimulating factor gene.

J Virol. 1998 Oct;72(10):8430-6.
PMID: 9733898; UI: 98406262

- ☐ **25:** Inchauspe G, Major ME, Nakano I, Vivitski L, Maisonnas M, Trepo C. Related Ar

Immune responses against hepatitis C virus structural proteins followi
genetic immunisation.

Dev Biol Stand. 1998;92:163-8.
PMID: 9554271; UI: 98214890

- ☐ **26:** Howard CR, Gray L, D'Mello F, Christopher J, Craske J. Related Ar

Nucleic acid vaccines against hepatitis viruses.

Dev Biol Stand. 1998;92:157-62.
PMID: 9554270; UI: 98214889

- ☐ **27:** Inchauspe G. Related Ar

Gene vaccination for hepatitis C.

Springer Semin Immunopathol. 1997;19(2):211-21. Review. No abstract available.

requested
122
11/6/00

PMID: 9406347; UI: 98069421

5

- ☐ 28: Geissler M, Gesien A, Wands JR.

Related A

Inhibitory effects of chronic ethanol consumption on cellular immune responses to hepatitis C virus core protein are reversed by genetic immunizations augmented with cytokine-expressing plasmids.

J Immunol. 1997 Nov 15;159(10):5107-13.

PMID: 9366440; UI: 98031768

- ☐ 29: Nakano I, Maertens G, Major ME, Vitvitski L, Dubuisson J, Fournillier A, De Martynoff G, Trepo C, Inchauspe G.

Related A

Immunization with plasmid DNA encoding hepatitis C virus envelope 1 antigenic domains induces antibodies whose immune reactivity is linked to the injection mode.

J Virol. 1997 Sep;71(9):7101-9.

PMID: 9261444; UI: 97404732

- ☐ 30: Inchauspe G, Major ME, Nakano I, Vitvitski L, Trepo C.

Related A

DNA vaccination for the induction of immune responses against hepatitis C virus proteins.

Vaccine. 1997 Jun;15(8):853-6.

PMID: 9234532; UI: 97378935

referred
12/11/6/00

- ☐ 31: Inchauspe G, Vitvitski L, Major ME, Jung G, Spengler U, Maisonnas M, Trepo C.

Related A

Plasmid DNA expressing a secreted or a nonsecreted form of hepatitis virus nucleocapsid: comparative studies of antibody and T-helper responses following genetic immunization.

DNA Cell Biol. 1997 Feb;16(2):185-95.

PMID: 9052739; UI: 97205235

- ☐ 32: Blum HE.

Related A

Update hepatitis A-G.

Digestion. 1997;58 Suppl 1:33-6. Review. No abstract available.

PMID: 9225087; UI: 97368473

- ☐ 33: Fomsgaard A.

Related A

[Genetic immunization--"the biological equivalent of cold fusion"]?

Ugeskr Laeger. 1995 Sep 4;157(36):4932-6. Review. Danish.

PMID: 7676526; UI: 95406976

- ☐ 34: Lagging LM, Meyer K, Hoft D, Houghton M, Belshe RB, Ray R.

Related A

Immune responses to plasmid DNA encoding the hepatitis C virus core protein.

J Virol. 1995 Sep;69(9):5859-63.

PMID: 7637033; UI: 95364007

Display	Summary	▼	Save	Text	Order	Details	Add to Clipboard
Show:	20	▼	Items 21-34 of 34		Page 2 of 2		Select page:

[Write to the Help Desk](#)
[NCBI](#) | [NLM](#) | [NIH](#)
[Department of Health & Human Services](#)
[Freedom of Information Act](#) | [Disclaimer](#)